

Dean L. Engelhardt, *et al.*

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Filed: June 7, 1995

Page 2 (Supplemental Amendment to Applicants' July 24, 1998

Supplemental Response and Their July 6, 1998 Amendment Under 37

C.F.R. §1.116 - February 2, 1999)



AMEND THE ABOVE-IDENTIFIED APPLICATION AS FOLLOWS:

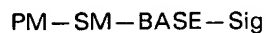
In The Claims:

Amend claims 284, 329, 331, 332, 333, 334, 335, 337, 348, 366, 367, 370 and 371 as follows:

284. (Four Times Amended) A process for detecting a nucleic acid of interest in a sample, which process comprises the steps of:

(a) hybridizing said nucleic acid of interest in the sample with an oligo- or polynucleotide comprising at least one nucleotide selected from the group consisting of:

(i) a nucleotide having the formula



wherein

PM is a phosphate moiety,

SM is a monosaccharide moiety,

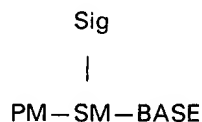
BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is a detectable moiety,

wherein PM is attached at the 3' or the 5' position of the monosaccharide moiety SM when said nucleotide is a deoxyribonucleotide and at the 2', 3' or 5' position when said nucleotide is a ribonucleotide, BASE is attached to the 1' position of SM from the N¹ position when BASE is a pyrimidine or the N⁹ position when BASE is a purine or a 7-deazapurine, and Sig is covalently attached to BASE at a position other than the C⁵ position when BASE is a pyrimidine, at a position other than the C⁸ position when BASE is a purine and at a position other than the C⁷ position when BASE is a 7-deazapurine and such covalent attachment does not substantially interfere with double helix formation or nucleic acid hybridization;

Enz-5(D8)(C2)

(ii) a nucleotide having the formula



wherein

PM is a phosphate moiety,

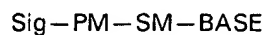
SM is a monosaccharide moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is a detectable moiety,

wherein PM is a phosphate moiety, SM is a monosaccharide moiety, and BASE is a pyrimidine, purine or 7-deazapurine moiety, said PM being attached to SM at a position independently selected from the 2', 3', and 5' positions of SM when said nucleotide is a ribonucleotide, and at a position independently selected from the 3' and 5' positions when said nucleotide is a deoxyribonucleotide, said BASE being attached to the 1' position of SM from the N¹ position when BASE is a pyrimidine or the N⁹ position when BASE is a purine or 7-deazapurine, and Sig is covalently attached to SM directly or through a linkage group and such covalent attachment does not substantially interfere with double helix formation or nucleic acid hybridization; and

(iii) a nucleotide having the formula



wherein

PM is a phosphate moiety,

SM is a monosaccharide moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sub
M1
K1
cont

Sig is a detectable moiety,
wherein PM is attached to the 3' or the 5' position of SM when said
nucleotide is a deoxyribonucleotide and at the 2', 3' or 5' position
when said nucleotide is a ribonucleotide, BASE is attached to the 1'
position of SM from the N¹ position when BASE is a pyrimidine or the
N⁹ position when BASE is a purine, and Sig is covalently attached to
PM and such covalent attachment does not substantially interfere
with double helix formation or nucleic acid hybridization; and

(b) detecting the presence of said detectable Sig moieties in any of the
oligo- or polynucleotides which have hybridized to said nucleic acid of
interest.

Sub
M2
K2

329. (Amended) A process for determining the sequence of a nucleic acid of
interest, comprising the steps of:

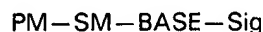
incorporating one or more modified nucleotides or an oligo- or
polynucleotide comprising one or more modified nucleotides into a nucleic acid or
nucleic acid fragments complementary to said nucleic acid of interest, wherein said
one or more modified nucleotides comprise a nucleotide modified on the sugar,
phosphate or base moieties thereof, and wherein said one or more modified
nucleotides are [self-signalling or] self-indicating [or self-detecting], to produce a
labeled nucleic acid or labeled nucleic acid fragments complementary to said
nucleic acid of interest or a portion thereof;

separating said labeled nucleic acid or labeled nucleic acid fragments in a
sequencing gel; and

detecting the presence of each [specific segment] of said separated labeled
nucleic acid or labeled nucleic acid [fragments] fragment by means of said [self-
signalling or] self-indicating [self-detecting] modified nucleotide or nucleotides.

331. (Twice Amended) The process according to [claim] claims 329 or 373, wherein said modified nucleotide comprises a member selected from the group consisting of:

- (i) a nucleotide having the formula



wherein

PM is a phosphate moiety,

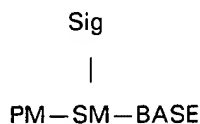
SM is a monosaccharide moiety,

BASE is a pyrimidine, purine 7-deazapurine, and

Sig is a detectable moiety,

wherein PM is attached at the 3' or the 5' position of the monosaccharide moiety SM when said nucleotide is a deoxyribonucleotide and at the 2', 3' or 5' position when said nucleotide is a ribonucleotide, BASE is attached to the 1' position of SM from the N¹ position when BASE is a pyrimidine or the N⁹ position when BASE is a purine or a 7-deazapurine, and Sig is covalently attached to BASE at a position other than the C⁵ position when BASE is a pyrimidine, at a position other than the C⁸ position when BASE is a purine, and at a position other than the C⁷ position when BASE is a 7-deazapurine [and such covalent attachment does not substantially interfere with double helix formation];

- (ii) a nucleotide having the formula



wherein

PM is a phosphate moiety,

SM is a monosaccharide moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

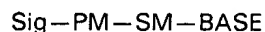
Sig is a detectable moiety,

said PM being attached to SM at a position independently selected from the 2', 3', and 5' positions of SM when said nucleotide is a ribonucleotide, and at a position independently selected from the 3' and 5' positions when said nucleotide is a deoxyribonucleotide, said BASE being attached to the 1'

Sub
M3
K3
cont

position of SM from the N¹ position when BASE is a pyrimidine or the N⁹ position when BASE is a purine or 7-deazapurine, and Sig is covalently attached SM directly or through a linkage group [and such covalent attachment does not substantially interfere with double helix formation]; and

(iii) a nucleotide having the formula



wherein

PM is a phosphate moiety,

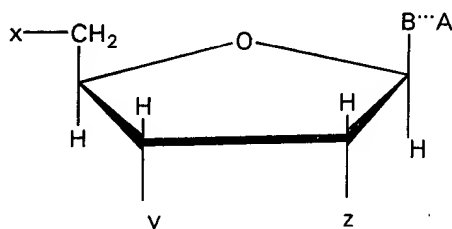
SM is a monosaccharide moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is detectable moiety,

wherein PM is attached to the 3' or the 5' position of SM when said nucleotide is a deoxyribonucleotide and at the 2', 3' or 5' position when said nucleotide is a ribonucleotide, BASE is attached to the 1' position of SM from the N¹ position when BASE is a pyrimidine or the N⁹ position when BASE is purine, and Sig is covalently attached to PM [and such covalent attachment does not substantially interfere with double helix formation].

332. The process according to [claim] claims 329 or 373, wherein said modified nucleotide has the structure:



wherein B represents a purine, a 7-deazapurine or a pyrimidine moiety suitable for incorporation into a polynucleotide and covalently bonded to the C¹-position of the monosaccharide moiety, provided that when B is a purine or 7-deazapurine, the

Sub
M3
K3
Concl'd

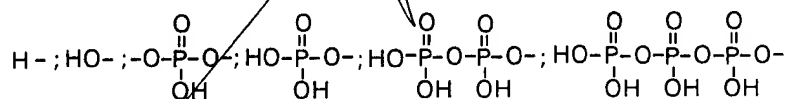
monosaccharide moiety is attached at the N⁹ position of the purine or deazapurine, and when B is a pyrimidine, the monosaccharide moiety is attached at the N¹ position of the pyrimidine;

wherein A represents at least three carbon atoms and is an indicator molecule that is [self-signaling or] self-indicating [or self-detecting selected];

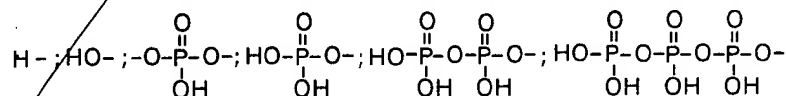
wherein B and A are covalently attached directly or through a linkage group, said linkage group not interfering substantially with detection of A;

wherein if B is a purine, A is attached to the 8-position of the purine, if B is a 7-deazapurine, A is attached to the 7-position of the deazapurine, and if B is a pyrimidine, A is attached to the 5-position of the pyrimidine; and

wherein x comprises a member selected from the group consisting of:



wherein y comprises a member selected from the group consisting of:



wherein z comprises a member selected from the group consisting of H- and HO-.

K4

333. (Amended) The process according to [claim] claims 329 or 373, wherein said [self signalling or] self-indicating [self-detecting] modified nucleotide comprises a member selected from the group consisting of a fluorescent component, a chemiluminescent component, and a chelating component, or a combination of any of the foregoing

Claim 334, line 1, after "claims 329 or" change "333" to -- 373 -- .

Claim 335, line 1, after "according to" change "claim 329"

to -- claims 329 or 373 -- .

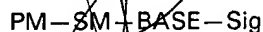
Sub
M4
X5

337. (Thrice Amended) A process for preparing a labeled oligo- or polynucleotide of interest, comprising the steps of:

(A) providing:

one or more chemically modified nucleotides capable of incorporating into an oligo- or polynucleotide, alone or in conjunction with one or more other modified or unmodified nucleic acids selected from the group consisting of nucleotides, oligonucleotides and polynucleotides, said other modified or unmodified nucleic acids being capable of incorporating into an oligo- or polynucleotide, said chemical modification comprising a label capable of providing directly or indirectly a detectable signal indicating the presence of said labeled oligo- or polynucleotide, said chemically modified nucleotides being modified on the sugar, phosphate or base moieties thereof and being selected from the group consisting of:

(i)



wherein

PM is a phosphate moiety,

SM is a monosaccharide moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

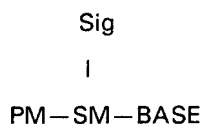
Sig is a detectable moiety, and

wherein PM is attached at the 3' or the 5' position of the monosaccharide moiety SM when said nucleotide is a deoxyribonucleotide and at the 2', 3' or 5' position when said nucleotide is a ribonucleotide, BASE is attached to the 1' position of SM from the N¹ position when BASE is a pyrimidine or the N⁹ position when BASE is a purine or a 7-deazapurine, and Sig is covalently attached to BASE directly or through a linkage group at a position other than the C⁵ position when BASE is a pyrimidine, at a position

Sub
M4
K5
cont

other than the C⁸ position when BASE is a purine, and at a position other than the C⁷ position when BASE is a 7-deazapurine [and such covalent attachment does not substantially interfere with double helix formation];

(ii)



wherein

PM is a phosphate moiety,

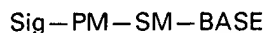
SM is a monosaccharide moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is a detectable moiety, and

wherein said PM is attached to SM at a position independently selected from the 2', 3', and 5' positions of SM when said nucleotide is a ribonucleotide, and at a position independently selected from the 3' and 5' positions when said nucleotide is a deoxyribonucleotide, said BASE is attached to the 1' position of SM from the N¹ position when BASE is a pyrimidine or the N⁹ position when BASE is a purine or 7-deazapurine, and Sig is covalently attached to SM directly or through a linkage group [and such covalent attachment does not substantially interfere with double helix formation]; and

(iii)



wherein

PM is a phosphate moiety,

SM is a monosaccharide moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is detectable moiety; and

wherein PM is attached to the 3' or the 5' position of SM when said nucleotide is a deoxyribonucleotide and at the 2', 3' or 5' position

when said nucleotide is a ribonucleotide, BASE is attached to the 1' position of SM from the N¹ position when BASE is a pyrimidine or the N⁹ position when BASE is purine, and Sig is covalently attached to PM directly or through a linkage group [and such covalent attachment does not substantially interfere with double helix formation]; and

said oligo- or polynucleotide of interest; and

(B) incorporating said one or more modified nucleotides into said oligo- or polynucleotide, thereby preparing a labeled oligo- or polynucleotide of interest.

348. (Thrice Amended) A process for detecting the presence of an oligo- or polynucleotide of interest in a sequencing gel, comprising the steps of:

(A) providing:

(a) one or more chemically modified nucleotides capable of incorporating into an oligo- or polynucleotide, alone or in conjunction with one or more other modified or unmodified nucleic acids selected from the group consisting of nucleotides, oligonucleotides and polynucleotides, said other modified or unmodified nucleic acids being capable of incorporating into an oligo- or polynucleotide, said chemical modification rendering said one or more chemically modified nucleotides either:

(I) [self-signaling or] self-indicating [or self-detecting]; or

(II) comprising a label capable of providing directly or indirectly a detectable signal;

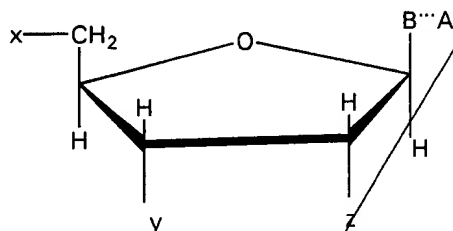
said [self-signaling or] self-indicating [or self-detecting] chemical modification or said label indicating the presence of said labeled oligo- or polynucleotide;

thereby indicating the presence of said labeled oligo- or polynucleotide, said chemically modified nucleotides being modified non-disruptively or disruptively on at least one of the sugar, phosphate or base moieties thereof; and

(b) an oligo- or polynucleotide;

(B) incorporating said one or more chemically modified nucleotides into said oligo- or polynucleotide, thereby preparing a labeled oligo- or polynucleotide of interest, said labeled oligo- or polynucleotide of interest comprising one or more chemically modified nucleotides selected from the group consisting of:

(i)

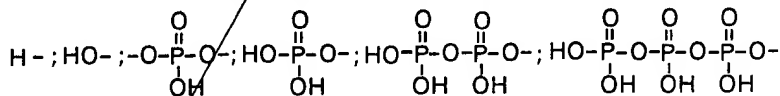


wherein B represents a purine, a 7-deazapurine or a pyrimidine moiety covalently bonded to the C1'-position of the sugar moiety, provided that whenever B is a purine or 7-deazapurine, the sugar moiety is attached at the N9-position of the purine or 7-deazapurine, and whenever B is a pyrimidine, the sugar moiety is attached at the N1-position of the pyrimidine;

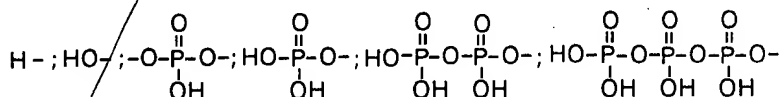
wherein A comprises at least three carbon atoms and represents at least one component of a signaling moiety capable of producing directly or indirectly a detectable signal or being [self-signaling or] self-indicating [or self-detecting]; and

wherein B and A are covalently attached directly or through a linkage group, and

wherein x comprises a member selected from the group consisting of:



wherein y comprises a member selected from the group consisting of:



wherein z comprises a member selected from the group consisting of H- and HO-;

(ii)

Sig

I

PM—SM—BASE

wherein

PM is a phosphate moiety,

SM is a monosaccharide moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is a detectable moiety or is self-indicating, and

wherein said PM is attached to SM at a position independently selected from the 2', 3', and 5' positions of SM when said nucleotide is a ribonucleotide, and at a position independently selected from the 3' and 5' positions when said nucleotide is a deoxyribonucleotide, said BASE is attached to the 1' position of SM from the N¹ position when BASE is a pyrimidine or the N⁹ position when BASE is a purine or 7-deazapurine, and Sig is covalently attached to SM directly or through a linkage group; and

(iii)

Sig—PM—SM—BASE

wherein

PM is a phosphate moiety,

SM is a monosaccharide moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is detectable moiety or is self-indicating; and

wherein PM is attached to the 3' or the 5' position of SM when said nucleotide is a deoxyribonucleotide and at the 2', 3' or 5' position when said nucleotide is a ribonucleotide, BASE is attached to the 1' position of SM from the N¹ position when BASE is a pyrimidine or the N⁹ position when BASE is purine, and Sig is covalently attached to PM directly or through a linkage group;

(C) transferring said labeled oligo- or polynucleotide of interest to a sequencing gel;

(D) separating said labeled oligo- or polynucleotide of interest from other nucleic acids not of interest; and

Enz-5(D8)(C2)

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Page 13 (Supplemental Amendment to Applicants' July 24, 1998

Supplemental Response and Their July 6, 1998 Amendment Under 37
C.F.R. §1.116 - February 2, 1999)

side
to
conclude
(E) detecting directly or indirectly the presence of said labeled oligo- or polynucleotide.

KN 366. (Amended) The process of claim 348, wherein the labeled oligo- or polynucleotide of interest prepared by said incorporating step comprises at least one [external] terminal modified nucleotide.

367. (Amended) The process of claim 348, wherein the labeled oligo- or polynucleotide of interest prepared by said incorporating step comprises at least one internal modified nucleotide and at least one terminal modified nucleotide.

R8 370. (Amended) The process of claim 348, wherein said direct detection is carried out on one or more [self-signaling or] self-indicating [or self-detecting] nucleotides.

371. (Amended) The process of claim 370, wherein said one or more [self-signaling or] self-indicating [or self-detecting] nucleotides comprise fluoresceinated nucleotides.

Add new claims 373-375 as follows:

Sub
M6
K9
373. (NEW) A process for determining the sequence of a nucleic acid of interest, comprising the steps of:

providing or generating a labeled nucleic acid or labeled nucleic acid fragments complementary to said nucleic acid of interest or a portion thereof, said labeled nucleic acid or labeled nucleic acid fragments being self-indicating and comprising one or more modified nucleotides modified on the sugar, phosphate or base moieties thereof;

introducing said labeled nucleic acid or labeled nucleic acid fragments into a sequencing gel;

separating said labeled nucleic acid or labeled nucleic acid fragments in said sequencing gel;

detecting the separated labeled nucleic acid or labeled nucleic acid fragments; thereby determining the polynucleotide sequence from the labeled nucleic acid or labeled nucleic acid fragments detected.

374. (NEW) The process of claim 373, wherein said detecting step comprises localizing said labeled nucleic acid or said labeled nucleic acid fragments by means of said self-indicating nucleotide or nucleotides.

375. (NEW) The process of claim 329, wherein said detecting step comprises localizing said labeled nucleic acid or said labeled nucleic acid fragments by means of said self-indicating nucleotide or nucleotides.

* * * * *